

ECOGENETICS OF SHELL SCULPTURE IN *ONCOMELANIA* (GASTROPODA) IN CANALS OF HUBEI, CHINA, AND RELEVANCE FOR SCHISTOSOME TRANSMISSION

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ABSTRACT

Oncomelania hupensis in China is well known as the intermediate host of the human blood parasite *Schistosoma japonicum*. There are three subspecies on the mainland of China with discrete patterns of distribution: above the Three Gorges of the Yangtze River (*O. h. robertsoni*) in Yunnan and Sichuan provinces; below the Three Gorges along the Yangtze River drainage (*O. h. hupensis*) with an incursion into Guangxi Province; and Fujian Province along the coast (*O. h. tangi*). Of these taxa, only *O. h. hupensis* has ribbed shells. Until now, *O. h. hupensis* has been shown to be dimorphic, with ribbed-shelled aggregates of individuals on flood plains and smooth-shelled populations in habitats elevated above the effects of floods or removed from the effects of severe annual floods by barriers. Molecular population genetics and anatomical studies have shown that there are no significant genetic differences between the two *O. h. hupensis* morphs; they belong to the same species (Davis et al., 1999b; Shi et al., 2002). Evidence to date has also shown that the ribbed-shelled aggregates of individuals are not true populations and are highly susceptible to infection with the parasite, whereas smooth-shelled populations have lesser potential to be infected, grading to total resistance.

We recently found in two canals of Hubei, well buffered from the annual Yangtze River floods, isolated populations that are truly polymorphic, with three to five classes of shell sculpture. The two canals were significantly different in their polymorphisms in 2001 (single sample per canal) and in 2004 (multiple samples within canals). We know the history of the construction of these canals (14 and 21 years ago, respectively), and the only available pathway of colonization of these canals (from the Yangtze River through the Guan Yin flood gate into the primary Hong Chou Canal). The colonizing snails were most probably derived from strongly ribbed snails of the adjacent flood plains. The changes from heavily ribbed to nearly smooth had to occur within the short span of 14 to 21 years. There were significant differences within canals in 2004 when multiple samples were taken.

The purpose of this paper is to present base-line data on this first reported case of shell sculptural polymorphism within *O. h. hupensis*, with the hypothesis that in the absence of severe flooding selection, this taxon will rapidly change from heavily-ribbed shells to slightly-ribbed to the smooth-shelled condition. Further, these changes give insight into questions of population evolution and coevolution with *Schistosoma japonicum*, in which smoothness is associated with genetic stability (defined in Davis, 1999a) that leads to the reduced potential to transmit the parasite (under coevolutionary pressure) and, in some instances, the evolved refractiveness to transmission.

Key words: schistosomiasis, *Schistosoma japonicum*, China, polymorphism, *Oncomelania*, evolution, population structure, coevolution, Red Queen.

INTRODUCTION

Oncomelania hupensis in China is well known as the intermediate host for the blood

fluke *Schistosoma japonicum* afflicting man and other mammals. There are three subspecies with discrete patterns of distribution on the mainland of China (reviewed in Davis,

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1992, 1994; Davis et al., 1995, 1999a). All three transmit *Schistosoma japonicum* strains. *Oncomelania hupensis hupensis* is found throughout the Yangtze River drainage system below the Three Gorges of the river. *Oncomelania h. robertsoni* is located in the highlands and mountains of Yunnan and Sichuan provinces above the Three Gorges. *Oncomelania h. tangi* lives in coastal areas of Fujian Province, isolated from the Yangtze River by a mountain range.

Oncomelania with its two species, and *O. hupensis* with several subspecies distributed in Japan, Philippines, Celebes, Taiwan, are taxa that, with one exception, have smooth shells, as seen in the other genera of the family Pomatiopsidae (Davis, 1979, 1980, 1992; Davis et al., 1999a). The exception is found in populations of *O. hupensis hupensis* of the flood plains of the Yangtze River and its tributaries. Such flood-challenged populations have prominent ribs. Additionally, flood-plain *O. h. hupensis* has longer, heavier shells than the other subspecies, especially *O. h. robertsoni*, with very small shells and no varix (special thickening of the outer shell lip).

Until now, *O. h. hupensis* has been shown to be dimorphic, with ribbed-shelled aggregates of individuals on flood plains and smooth-shelled populations in habitats elevated above the effects of flooding. Molecular population genetics and anatomical studies show that there are no significant genetic differences between the smooth-shelled and ribbed-shelled populations; they belong to the same subspecies (Davis et al., 1995, 1999b, using allozymes; Shi et al., 2002, using mitochondrial CO1 gene sequencing). Based on breeding genetics, ribbing in *Oncomelania hupensis* is controlled by a single locus (Mendelian inheritance of a single gene) where ribs

are dominant, smooth recessive, and with multiple alleles of that gene (Davis & Ruff, 1973). Likewise, size is controlled by alleles at a single locus.

All evidence indicates that ribbing is an evolved response to heavy annual flooding, that is, ribbing is maintained by natural selection. The hypothesis is that increased size and ribbing of flood plain individuals confer a selective advantage by way of strengthening the shells and enabling flotation to survive flooding (reviewed in Davis et al., 1999a, b).

We are currently studying the ecogenetics of *Schistosoma* transmission in two selected inner "tertiary" canals of Hubei, because they have an environment that is the most buffered from the ravages of the annual floods of the Yangtze River. Additionally, numbers of these canals, at the same or lower elevation as the Yangtze River, are relatively recently constructed and thus provide an opportunity to study a number of unique factors impacting disease transmission.

In October 2001, while selecting study sites, we found populations of *Oncomelania hupensis hupensis* in two unconnected canals, 2.7 km apart, that had shells that were polymorphic for ribbing. These canals were chosen because they are part of a *Schistosoma japonicum* endemic area, with infected snails in these canals, and the canals are far removed from the influence of the Yangtze River. We scored shells from a single population from each canal for strength of ribbing and found that three to four classes of ribbing could be identified. Further, the populations had significantly different frequencies of the morphs (Table 1, $P > 0.0001$).

The purpose of this paper is to present initial base-line data derived from analysis of shell ribbing in populations along these two Hubei canals (both the 2001 and 2004 data), and to demonstrate that within populations there are polymorphisms that we hypothesize to be stages of loss of ribbing in the absence of flooding selection. Further, the polymorphisms found give insight into questions of population evolution and coevolution involving (1) the timing of reversion from ribbing to smoothness; (2) the coevolution of *Oncomelania hupensis hupensis* with *Schistosoma japonicum*, in which smoothness is associated with genetic stability (defined in Davis et al., 1999a) leading to the reduced potential to transmit the parasite, and in some situations, the evolved refractiveness to transmission.

TABLE 1. χ^2 comparison of shell polymorphisms of shell ribbing on *Oncomelania hupensis hupensis* snails from two Hubei Canals in 2001. N = number of shells from living snails. Data given as % of N. $P > 0.0001$. See text for details.

	Ma Ling (N = 101)	Gu Hu (N = 105)
M	13.9	43.8
SL	61.3	40.0
S-/S	24.8	16.2

METHODS

Canal Locations and Descriptions

The canals are located in the administrative villages of Gu Hu and Ma Ling of Sha Shi District, Jingzhou City, Hubei. The Gu Hu Canal ($30^{\circ}19.129'N$, $112^{\circ}23.386'E$ at mid-canal) is 1.6 km long. It is oriented E–W (95° – 275° true). The Ma Ling Canal ($30^{\circ}19.578'N$; $112^{\circ}21.270'E$ at mid-canal) is 1.48 km long, with a N–S orientation (187° – 8° true). Both canals are absolutely straight. We divide the Ma Ling Canal into two zones, the dividing point being where the canal is interrupted by a wider canal, the Wu Yi Canal. The right angle intersection is open in all four directions. Water from the Ma Ling–Wu Yi juncture flows through a pipe under the road, which parallels the Wu Yi Canal, to flow N 1 km to dead-end at the end of zone 1 of the Ma Ling Canal. At high water, this northern zone acts as a drainage canal. If water levels get too high, water is pumped from the northern end to the immense Si Hu Canal on the other side of a high dyke. The Si Hu, built pre 1960, is a major drainage canal flow-

ing east. Zone 2 runs south of the Wu Yi Canal and at low water it is separated from the Wu Yi Canal by a very low, man-made earthen dam that holds back water of zone 2 to form a duck pond. Further south, the standing water meanders between low banks with thick marsh grass providing an ideal marshy environment for snails. Zone 2 is about 280 m long.

Both canals are about 4 to 5 m wide. The canals are separated by 2.7 km, with the Gu Hu Canal due E from the Ma Ling Canal. The unconnected canals are 2.4 km S of the vast Chang Lake and 12 km E of the Yangtze River. The canals in question are less than 22 years old. The Ma Ling Canal was built in 1984 and Gu Hu Canal in 1992.

Sampling and Scoring

Polymorphism data were an unintended and surprising byproduct of the primary purpose of our research on the long-term consequences of environmental change on the genetics and infectivity patterns of snails in recently constructed and highly protected canal systems. Data were taken from the 2001 mass collec-

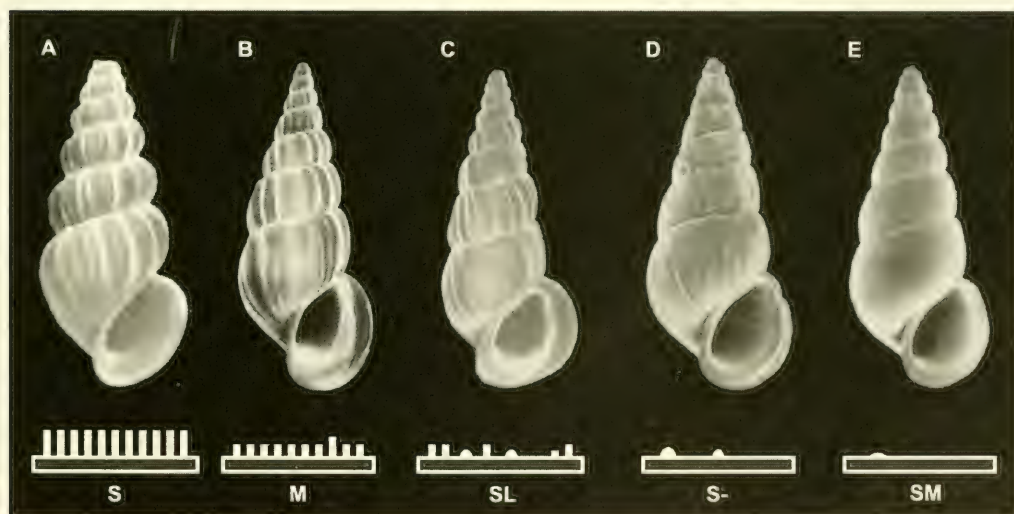


FIG. 1. Degrees of shell ribbing of *Oncomelania hupensis hupensis*. The schematic drawings (below) make clear the degree of ribbing strength and height that are very difficult to adequately portray in photographs or SEM pictures of shells (above). A: Strong ribs (S); B: Medium ribs (M); C: Slightly ribbed (SL); D: Trace of ribbing (S-); E: Smooth (SM). In D and E the "bumps" on the shell may be low swellings of a growth line or slightly elongated very low nodes. In smooth shells, the shell may be entirely smooth or, in few individuals, there may be one or two very low nodes or swellings indicating a highly degraded rib.

tion of snails taken to see if the sites were fitting for future study and from the experimental study initiated in 2004.

For our primary purpose, snails were collected each collection period (twice a year) from a 2 m² (a half frame, see Davis et al., 2002) positioned at 20 randomly selected sites on either side of the canal, enabling estimates of snail density per m² for each canal. Where there were ten or more snails per 4 m² (a frame) or ≥ 2.5 snails per m², (snails pooled from both sides of the canal) there were sufficient snails to score shells for one of five possible shell sculpture conditions (Figs. 1A–E): smooth (SM), smooth with negligible trace of ribs (S-), slightly ribbed (SL), medium ribbed (M), and strongly ribbed (S). Strong ribs are the type of ribbing found on the Yangtze River flood plains (heavy shells, tall and thick ribs regularly positioned on each whorl).

Of the 40 sites sampled, only five had ten or more snails per frame. Smooth shells vary from having a completely smooth shell surface to one which may have slight irregularities as a low swelling (bump) or an irregular growth line Figure 1E. Between these extremes (smooth or heavily ribbed) are three intermediate conditions (1) Negligible ribbing (Fig. 1D): the shell surface varies from completely smooth to having one or two scattered irregular nodes, or low thin rib lines on the body and penultimate whorl indicating the position where a rib might develop. (2) Slightly ribbed (Fig. 1C): The shell surface has some irregularly placed low, thin ribs with some rib-nodes (undeveloped ribs). (3) Medium ribbed (Fig. 1B): The penultimate and body whorls have regularly positioned fully developed low ribs on the entire whorls. These ribs are considerably lower than those found on heavily ribbed shells.

Statistical Analysis

Microsoft Excel was used for X² analyses of morph frequencies. Given the small numbers in some cells, the Fisher Exact Test was used.

RESULTS

Results of Initial 2001 Exploratory Canal Examinations

On 25 October 2001, we collected snails from the first 1/3 of each canal closest to the main road. The snails were collected from a small area, about 90 m² along one side of each canal for the purpose of seeing what they looked like and if they were infected. Examination with a dissecting microscope at relatively low power showed the shells to have different patterns of ribbing. We could easily discern four types that we classified at that time as (1) smooth to trace of ribs (= types D and E, Fig. 1), (2) slightly ribbed, (3) medium ribbing. There were no strongly ribbed shells. The results of the scoring and the X² analysis given in Table 1 show a highly significant difference between the canals (P > 0.0001). The Fisher Exact Tests did not change the results. Ma Ling had significantly more slightly ribbed shells and significantly fewer medium ribbed shells than Gu Hu. This was the first time, to our knowledge, that such polymorphisms within populations of *Oncomelania* were found to exist.

Results of the September 2004 Collection

Scores for the four Ma Ling and one Gu Hu sites are given in Table 2 both for actual numbers of snails scored and % of snails in each

TABLE 2. Scoring snails for all sites for number and % snails with each morph category. S = strong ribs; M = medium ribbing; SL = slightly ribbed; S- = trace of ribbing; SM = smooth.

	Ma Ling				Gu Hu
	Site 1 (N = 15)	Site 17 (N = 12)	Site 19 (N = 122)	Site 20 (N = 96)	Site 16 (N = 77)
S	0	0	2 (1.6%)	0	0
M	15 (100%)	1 (8.3%)	31 (25.4%)	39 (40.6%)	38 (49.4%)
SL	0	10 (83.3%)	74 (60.7%)	52 (54.2%)	37 (48.1%)
S-	0	1 (8.3%)	14 (11.5%)	5 (5.2%)	2 (2.6%)
SM	0	0	1 (0.8%)	0	0

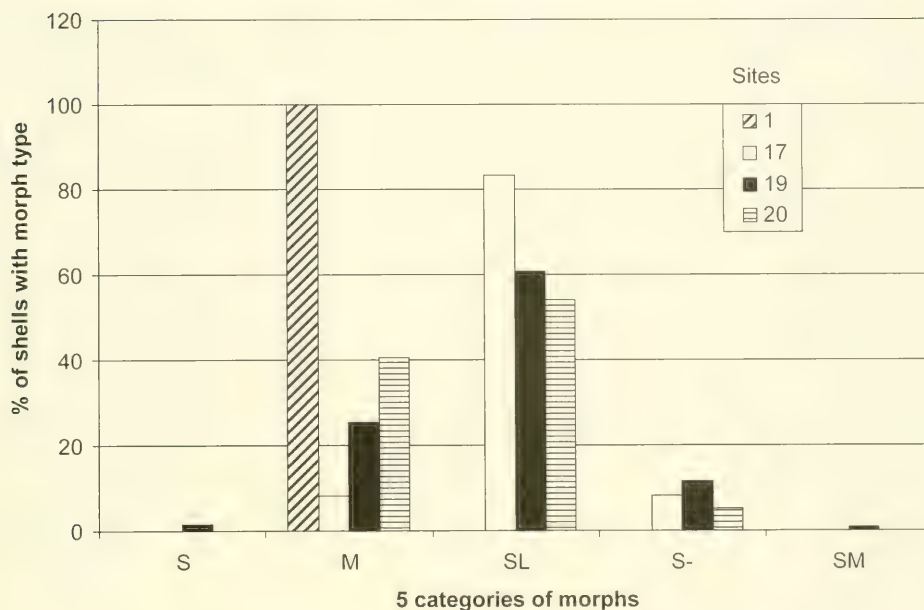


FIG. 2. The percent of morph types in the Ma Ling sites. S = strongly ribbed; M = medium ribbing; SL = slightly ribbed; S- = trace of ribbing; SM = smooth.

of the five morph classes. The percentage of each morph type in the four Ma Ling sites is graphed (Fig. 2). Ma Ling site one, at the northern end of the canal (zone 1, 1.0 km from site 17 close to the Wu Yi intersection), was unique

in having only medium-ribbed shells, but the number of snails collected (15) was low. The remaining sites were separated by distances ranging from 25 m to 50 m between them. Snails from site 17 (zone 1 close to the Wu Yi

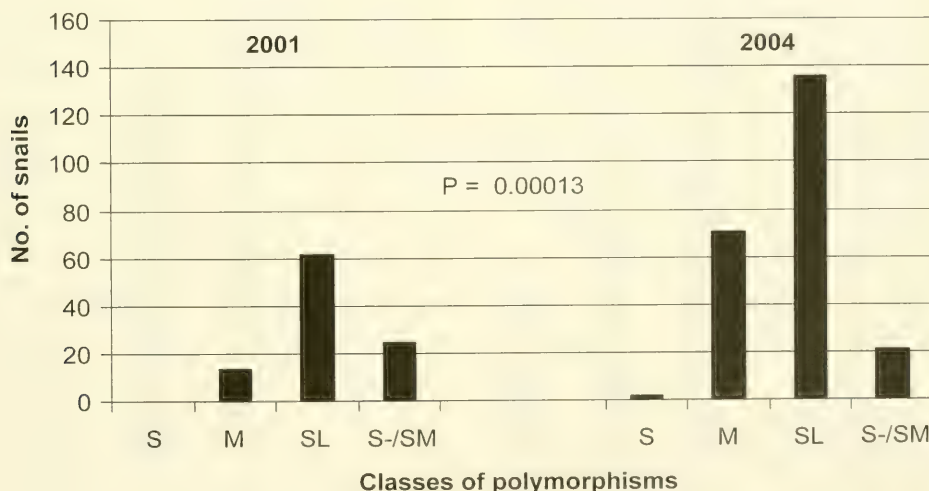


FIG. 3. Comparing Ma Ling snails from 2001 and 2004 (sites 17, 19, 20 combined data) for numbers of snails in each of four shell ribbing classes. See Fig. 2 for abbreviations. X^2 = highly significant difference.

TABLE 3. Cross comparison of canals and localities for years 2001 and 2004 to determine level of significant differences among sites for polymorphic classes of shell sculpture. **NS** = not significant; **BNS** = barely not significant; **HSD** = highly significant difference; **SD** = significant difference; **VSD** = very significant difference.

	2001 Ma Ling	2001 Gu Hu	2004 Gu H	2004 Ma Ling 1	2004 Ma Ling 17	2004 Ma Ling 19	2004 Ma Ling 20
2001 Ma Ling	-	HSD	HSD	HSD	NS	SD	SD
2001 Gu Hu		-	SD	HSD	SD	VSD	SD
2004 Gu Hu			-	HSD	SD	VSD	NS
2004 Ma Ling 1				-	HS	HSD	HSD
2004 Ma Ling 17					-	NS	NS
2004 Ma Ling 19						-	BNS (0.060)
2004 Ma Ling 20							-

canal intersection) were unique in having 83% slightly ribbed shells (but again low numbers, i.e., 12). Sites 19 and 20 were in zone 2. A cross comparison of sites for significant differences

(Table 3) yielded only five comparisons that were not significantly different (or barely not significantly different. Sites at the southern end of the Ma Ling Canal in 2004 (sites 17, 19, 20)

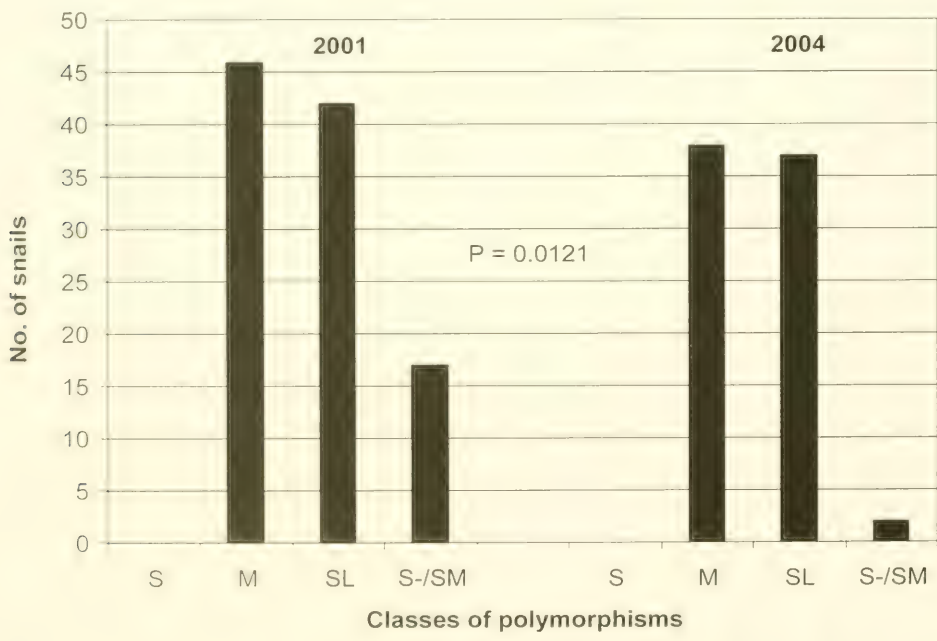


FIG. 4. Comparing Gu Hu snails from 2001 and 2004 for shell polymorphisms. See Fig. 2 for abbreviations. χ^2 = significant difference but barely so.

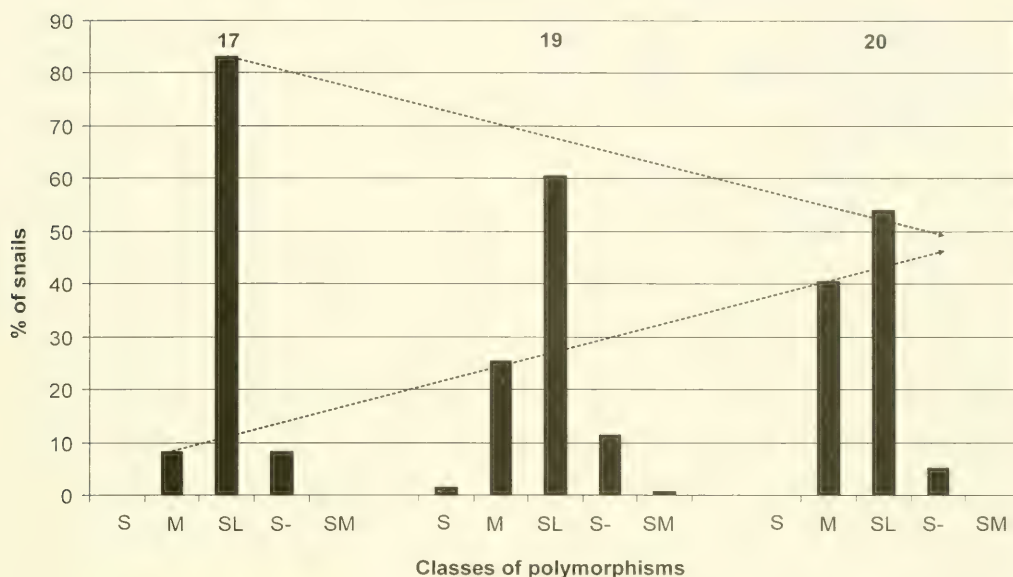


FIG. 5. Comparing Ma Ling 2004 sites 17, 19, 20 for five classes of shell ribbing polymorphisms. The % of each class is shown. See Fig. 2 for abbreviations. Dashed arrows indicate trend for decreasing slightly ribbed moving up the canal and increasing medium ribbed snails.

were not significantly different or bordering not significantly different.

To enable cross comparisons of 2001 and 2004 data, we combined data S- and SM from 2004. We also combined data from Ma Ling 17, 19, and 20, as 2004 populations were either not significantly different or barely significantly different. The Ma Ling snails from 2001 were highly significantly different from those of 2004 (Fig. 3). The 2004 population had fewer S- class and more M class snails. The Gu Hu snails from 2001 and 2004 were significantly different but barely so (Fig. 4). Most noticeable was the decrease in 2004 of S- class snails.

In comparing Ma Ling southern canal populations (17, 19, 20), one notices two distinct trends. Moving from north to south and across the larger perpendicular canal, there is distinct decrease in percentage of snails of the slightly ribbed class (Fig. 5). There is a distinct increase in snails of the medium-ribbed class.

Entirely smooth snails are thus far rare in these canals.

DISCUSSION

The major findings of this study are as follows. (1) For the first time shell sculptural polymorphism at a single site is reported in *On-*

comelania. (2) The polymorphism in degree of ribbing from strongly ribbed to smooth-shelled individuals in a relatively new canal environment is not static. Changes occur over a short period of time, that is less than 22 years, from strong ribbing to reduced strength of ribbing, including loss of ribbing. Further, morph frequency changes have occurred over the short span of 2–3 years. (3) The occurrence of shell sculpture polymorphism in isolated populations provides the first demonstrable linkage between ribbed-shelled and smooth-shelled *O. hupensis hupensis* populations, the rate with which the transition from the ribbed to smooth states can occur, and enables a clearer understanding of population genetic structure and the potential for populations to transmit *Schistosoma japonicum*.

Shell Sculptural Polymorphism and Environmental Selection

Polymorphism here is apparently dependent on a relatively new man-made environment that is affecting the genetic structure of these populations. Stability and removal from the annual floods of the Yangtze are the keys to change. Individuals in these relatively isolated canal habitats are changing from the heavily ribbed morphotype seen along the banks of

the Yangtze River, where their recent ancestors originated. Ribbing, as a derived flood-induced character, must be an energetically expensive character to maintain, as it is lost so quickly once the environmental enforcement is removed.

These canals are low land environments. Flow of water into the canals from the Yangtze is controlled by flood gates. The origin of the canal snails in this large region must be from the Yangtze River flood plains, dispersing into canals as the canals were built. The flotation of living snails from the Yangtze through the river embankment portals has been well documented (Xu & Fang, 1988; Xu et al., 1989, 1993; Yang et al., 1992). The only plausible origin for these snails is from the Yangtze River through the Guan Yin flood Gate into the primary Hong Chou Canal, hence to the north trending secondary Nan Bei Canal. The Nan Bei Canal is piped under (reverse siphoning) the Huge Shi Gong (that takes waste water away from Sha Shi City) to flow north to Malin Village. The Wu Yi Canal turns west off of the Nan Bei Canal to transect the Ma Ling Canal of our study about a km away.

Now we see deep in the tertiary interior canals that there has been a considerable change over the past 20 or so years since these particular canals were made. In 2004, only 0.47% of the snails had strong ribs, while 41% had slightly irregularly ribbed shells, and 5% were close to smooth. The significant differences between canals and between years within a given canal demonstrate a dynamic process, such as seen in Figure 5 showing discrete trends of increasing and decreasing morph frequencies between relatively closely positioned sites. We do not know the reason(s) for these short-term differences (or trends). In these canals, snails have low vagility and are subject to regular perturbation by man and animals. Differences could be due to founder effects or local selective pressures of micro-area effects.

The compelling argument is that flooding selection drives the selection for alleles favoring development of stronger shells, larger size, and ribs. Predation does not appear to drive these genotypes. The only known predators of *Oncomelania hupensis* snails are ducks and perhaps some predatory fish. But in the presence of ducks, *Oncomelania* in the southern zone of the Ma Ling Canal do not have strong ribs; they mostly have slightly ribbed shells (> 50%) and there are many (9%) nearly smooth (S-) shells. It is unlikely that fish are a factor

as adult *Oncomelania* is amphibious, living in the ecotone between water and dry land, a habitat not accessible to fish.

Ecology, Population Genetics of *Oncomelania hupensis hupensis* and the Transmission of *S. japonicum*

While there is no direct genetic linkage between shell sculpture and the potential to transmit *Schistosoma japonicum*, there are significant differences between strongly ribbed-shelled aggregates of *O. hupensis hupensis* and smooth-shelled populations with regard to both population genetic structure and the potential to transmit *Schistosoma japonicum*. Empirical data have shown ribbed-shelled aggregates of snails to be both genetically unstable (thus not true populations) and highly susceptible to infection with *Schistosoma japonicum*. Smooth-shelled populations have this far been shown to be genetically stable and have low to no capacity to transmit *S. japonicum* (Davis et al., 1999a; Wilke et al., 2000; Shi et al., 2002). The infectivity capacity has been hypothesized to be driven through coevolution with *S. japonicum*, with infectivity differences the basis for invoking the Red Queen hypothesis of coevolution of Van Valen (1973) (Davis, 1980, 1992: 193).

Origin of Ribbing, Genetic Instability and Infectivity

Historically, the evolutionary developments have been: (1) The plesiomorphic state (primitive or basic state) is being small and with smooth shell (Davis, 1979). (2) With evolving river systems and dispersal down the Yangtze River of the smooth-shelled morph into the new environment of the evolving Yangtze River and onset of annual monsoon-floods, there evolved the presence of ribs, a thicker shell, and increased size to cope with environmental challenge. Of all *Oncomelania* taxa, only the Yangtze River drainage (and derived drainages) developed ribbing on the shells. (3) Below the Three Gorges of the Yangtze River and dispersing up into habitats not affected by flooding, as well as dispersing to Taiwan and Japan, the snails reverted to (or maintained) a smooth shell. (4) Becoming smooth and living in isolation from immigration enables genetic stability, a requirement for the Red Queen to operate. In isolation, and normal inbreeding, smooth-shelled individuals evolve under selection pressure of the parasite from

being highly susceptible to the parasite to lowered susceptibility at the population level to totally resistant to infection.

Genetic Structure and the Transmission of *S. japonicum*

Population genetic stability vs. instability was defined (Davis et al., 1999a) using MtDNA sequence data. Low haplotype diversity (1 or 2 haplotypes per ≥ 10 individuals collected from a small area (e.g., 100 m²) is a surrogate for Hardy-Weinberg equilibrium or normal panmixis within a population over a period of years, that is, stability. Instability is indicated by high haplotype diversity for the same conditions above, where 6–10 haplotypes are found in ≤ 10 individuals. Instability is indicative of aggregates of individuals ("populations") of recent immigration; that is, these are not part of a normal interbreeding population. Snails are swept together from different locations, carried by flood waters. Hardy Weinberg is not attained. The example was given (Davis et al., 1999a) where five "populations" around the shores of Dong Ting Lake, all subjected to severe annual flooding, had heavy ribbing. These had 6–10 haplotypes for ten individuals thus all were unstable. The sixth population came from an elevation between 100 and 500 m. The shells were smooth and had two haplotypes per ten individuals, that is, genetic stability. All the ribbed snail populations were highly susceptible to schistosome infection. The smooth-shelled population was not and could not be infected (Li, Hunan Institute of Parasitic Diseases, personal communication).

A study of *O. hupensis hupensis* populations along the Yangtze River from Hunan and Hubei provinces through to Zhejiang and Jiangsu provinces involved questions of population evolution, haplotype diversity and ecology (Wilke et al., 2000). The data indicated that ribbing is associated with annual floods along the flood plains of the Yangtze River, where snails are swept into aggregates of snails with high haplotype diversity. In areas not affected by flooding, the snails were generally smooth and genetic diversity decreased significantly. The one Jiangsu population was smooth and living essentially at sea level in a water network (one haplotype in six individuals). One Zhejiang population (elevation of 100 m) had a trace of ribbing and low haplotype diversity. One Anhui population living in the lowlands but potentially removed from flooding had slightly ribbed shells and three haplotypes per ten individuals.

A study re-visiting the Miao River (Shi et al., 2002) involved haplotype analysis and ecological setting to examine both the question of smooth-shelled vs. ribbed shelled and the relationship between these morphs and infectivity. There was a clear trend for decreasing haplotype diversity upstream from the mouth of the river. Nucleotide-sequence diversity was > 0.015 at sites A and B close to the mouth of the river and where the snails had heavily ribbed shells; it was < 0.0085 at site G at the top of the river, where the snails were smooth (sites above the flood level with smooth-shelled populations were D–G). With regard to infectivity, the down-stream ribbed-shelled "populations" had higher infection rates and higher susceptibility to infection with *S. japonicum* than did upstream smooth-shelled populations. The higher infectivity of down-stream "populations" was attributed to the importation and mixture of snails (i.e., aggregates) of different genotypes of snails and schistosomes in flooded areas increasing the possibility of multiple infections by schistosomes of different genotypes. In upstream populations, low infectivity is probably due to isolation and attaining equilibrium, with the parasite at low frequencies of infection.

The Red Queen and Decreasing Infectivity

The Red Queen pertains to co-evolution in which the impact of a parasite on the host (in this case the intermediate snail host) elicits a genetic response of the host to repel the parasite. This in turn generates a genetic response in the parasite to overcome the defense of the host. Through time, the interaction becomes highly specific and convoluted. For this to happen, the snail population must be, in fact, a true population with little or no immigration, that is, in Hardy-Weinberg equilibrium (genetically stable). There are three possible end results of this "genetic war". (1) The parasite wins, and the snail population goes extinct (witnessed in the decline and local extinction of *Hydrobia truncata* in New England, USA (Davis et al., 1988). (2) The snail wins, and the parasite becomes extinct in the snail population. (3) The snail infectivity rate decreases until some equilibrium is reached at a low level of infectivity.

We have uncovered a number of situations in which the smooth-shelled snail population has gone to fixation for completely warding off the parasite. Davis & Ruff (1973) hybridized smooth totally refractive *Oncomelania hupen-*

sis from Taiwan with highly susceptible ribbed-shelled *O. hupensis* from the mainland of China. The hybrids could be infected; there was indeed a genetic component to transmission. On Taiwan, there are *Oncomelania hupensis* populations that are totally refractive, others are susceptible to non-human *Schistosoma japonicum*, and one population is not naturally infected with any schistosome but can be infected with all allopatric strains of *S. japonicum*.

We have found one Anhui smooth-shelled population that is totally refractive to infection. The refractive population above Dong Ting Lake was mentioned. The Miao River study demonstrated the highly infectious nature of the genetically unstable downstream ribbed-shelled snails in contrast to the much less susceptible upstream smooth-shelled populations.

There is a large literature on cross infectivity studies, based on the pioneering work of DeWitt (1954) involving permutations and combinations of *S. japonicum* from different localities and countries and *Oncomelania hupensis* from the corresponding localities. A sampling of a few papers on cross susceptibility studies are Moose & Williams (1963), Chi et al. (1971), He et al. (1991), Lin et al. (1994), Hong et al. (1995), and Sheng et al. (1995). These studies show that there is considerable evidence for evolutionary divergence among allopatric populations with regard to the genetic potential to transmit an allopatric *S. japonicum*. The results range from complete incompatibility to partial compatibility involving allopatric pairs.

We continue to maintain the hypothesis that genetically unstable aggregates of ribbed-shelled snails are more highly susceptible than isolated populations of smooth-shelled snails because the mixing of snails, due to importation by flooding (with an array of genotypes relative to schistosome success or failure at infecting these snails), facilitates high success in infecting snails. In such an environment, the schistosomes have a "menu" of genotypes to choose from with regard to their success in infecting the snail. Given the unstable nature of the mixture of the moment, one sees little opportunity for selective pressures to act on these genotypes relative to the process of speciation or emerging new disease. However, the mixture is a potent cocktail of genotypes that present a dangerous situation relative to importing the mixture, with all its genetic diversity, to a new environment. In genetically stable populations that are isolated and where

the Red Queen is in action, it is possible that selective pressures on allopatric stable populations could drive speciation and emerging disease.

Oncomelania hupensis robertsoni – A Different Evolutionary Trajectory

Oncomelania hupensis robertsoni has a different history and involvement with *Schistosoma japonicum* than *O. h. hupensis*. *Oncomelania hupensis robertsoni* is highly divergent genetically from *O. h. hupensis* (Davis et al., 1998; Wilke et al., 2000, 2006). As described above, this taxon, living in the mountains of Sichuan and Yunnan provinces, closer to the area of origin of the genus than *O. hupensis hupensis*, is not affected by the great annual floods of the Yangtze River, and has small, smooth shells and no varix. The varix, the thickening of the outer lip seen in *O. h. hupensis*, is equal to the terminal rib seen in all *O. hupensis hupensis* populations, smooth or ribbed. Of note is that thus far no population of *O. h. robertsoni* has been found to be refractive to infection with the Yunnan-Sichuan strain of *S. japonicum*. A different dynamic seems to be at work here. It is also noted that *robertsoni* lives on the banks of small streams, irrigation ditches, and the base of retaining walls of agricultural terraces. These are generally in a high gradient environment, where heavy rain wash snails downward. Following such rains, the snails, which are negative geotropic and negatively rheotropic, move relentlessly upward. The net result is hypothesized to be considerable genetic mixing within a drainage system, that is, resulting in genetically unstable aggregates of snails with high susceptibility to *S. japonicum*. Preliminary data support the instability argument as of 13 populations studied in Wilke et al. (2006), 40 of 66 specimens had different haplotypes, and of 24 specimens studied, each had a unique AFLP fingerprint. These data indicate a lack of population structure due to great heterogeneity, not due to uniform panmixia.

A great deal of work must be done with *O. h. robertsoni* on population genetics, population structure, natural patterns of infection, and laboratory infectivity studies before one can say much more. A tentative hypothesis is that *robertsoni* maintains the plesiomorphic receptiveness to infection and those environmental-population factors do not promote the Red Queen.

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